ZOOLOGICAL SCIENCE 8: 207–210 (1991)


[COMMUNICATION]

# Active Component of the Contraction Factor on Smooth Muscle Contraction of Gonad Wall in Sea Urchin

Nobuaki Takahashi[1,2], Noriyuki Sato[2,4], Yoichi, Hayakawa[3], Masaki Takahashi[1], Hirotsugu Miyake[1] and Kokichi Kikuchi[2]

[1]*Marine Biomedical Institute, Sapporo Medical College, Higashirishiri, Hokkaido 097-01,* [2]*Department of Pathology, Sapporo Medical College, Sapporo 060, and* [3]*Biological Laboratory, The Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan*

**ABSTRACT**—The present study analyzes active component of (a) contraction factor (CF) produced by aboral intestine for contraction of the sea urchin gonadal smooth muscles. The CF-I was previously reported to be present within the heamal vessel and to be a glycoprotein with a molecular weight of 3,800. The present investigation has documented that the active component of CF-I is a carbohydrate portion which is able to induce the contraction.

## INTRODUCTION

Our previous report [1] demonstrated that there exists (a) gonadal contraction factor (CF) within the heamal vessel of the sea urchin by immunohistochemically analyzing with a monoclonal antibody, #11-B-2, against the CF. Moreover, it was documented that the CF has a molecular weight of 3,800. Half a century ago, Palmer [2] took notice of the presence of gonadal contraction factor(s) but its localization and chemical nature remained to be determined. Nowadays, a monoclonal antibody against the CF has solved some part of those problems, as seen in detail in our data [1]. Moreover, the present study dealts with analysis of active component of the CF.

Accepted June 20, 1990
Received September 11, 1989
[4] To whom all correspondence should be addressed.

## MATERIALS AND METHODS

### *Animals*

Sea urchin (*Strongylocentrotus intermedius*) was used for a bioassy of the contraction.

### *Seawater*

Modified Van't Hoff seawater (ASW) (462 mM NaCl; 9 mM KCl; 9 mM $CaCl_2$; 63 mM $MgCl_2$; 17 mM $MgSO_4$; 20 mM Tris-HCl, pH 8.2) was used as a basal incubation medium.

### *Chemicals*

Our previous study [1] indicated that the CF is composed of a glycoprotein. To examine whether the active component within a moleculre of CF is localized in the peptide or carbohydorate portion, the experiment of proteolysis and glycolysis was performed.

Trypsin (5 mg/ml), pronase E (200 $\mu$g/ml), papain (45 units/ml), pepsin (1 mg/ml), carboxpeptidase A-PMSF (90 units/ml), neuraminidase (10 mU/ml), cellulase (1 mg/ml), $\alpha$-glucosidase (1 mg/ml), and RNase (1 mg/ml) were respectively mixed with 1 ml of crude water-extract of aboral intestine (2.5 mg/ml) or active CF-I fraction obtained in gel filtration [1] and incubated for 2 hr at 37°C. The mixtures were sometimes vigorously

shaken. Those emzymatic activities were stopped by treating with boiling water for 15 min. Thereafter, the solution containing the CF was diluted ten times with ASW of a higher concentration (10/9 times) and the contraction activity was measured. Incubation with trypsin, cellulase and $\alpha$-glycosidase was also performed for overnight. Chemicals were purchased from Sigma Chemical Company, except for pronase E (Kaken Kagaku, Tokyo).

*Recording and bioassay* [1]

To measure gonad contraction, a hole 3 cm in diamter was made on the oral side of each sea urchin with solid scissors. The animal was subsequently fixed by pinching the test with large forceps and immersed in a beaker up to its equator in filtered natural seawater. The body cavity was then filled with ASW. The straw previously cut vertically in half and connected to a strain guage (SB-1TH, Nihon Koden) was placed on a gonad. The siphon was situated in the central portion of the body cavity. The gonad was allowed to remain in this state for 30 min. The gonad was first treated with ASW containing CF until the time immediately following a peak or induced contraction. The gonad was then washed, and placed in ASW solution. The bioassy against one sea urchin was performed about ten times.

## RESULTS

Among the two contraction factors (CF-I and -II) [1] CF-I appeared in *S. intermedius* at maturation period and was mainly present within heamal vessel. Then, out study focused on the CF-I species. CF-I recognized by #11-B-2 monoclonal antibody [1] was 3,800 in molecular weight, and seemed to be glycoprotein, for it was stained by both coomassie blue and Shiff's reagent in Swank and Munkres gel [1]. Does either glyco- or protein-component of CF induce the contraction activity? This point was examined by proteolysis and glycolysis of the CF-I fraction.

It was first examined whether the protein component of CF was responsible for induction of the contraction. Enzymes were examined at several concentrations such as 5 mg/ml of trypsin, 200 $\mu$g/ml of pronase E, 1 mg/ml of pepsin, 90 units/ml of carboxypeptidase, and 45 units/ml of papain. Enzymes (1 ml each) were mixed with 1 ml of crude water-extract of the aboral intestine (2.5 mg/ml) or CF-I fraction obtained by Sephadex G-25 gel filtration and the mixtures were incubated for 2 hr, at 37°C. Subsequently, enzymes were inactivated by heat-treatment, which is known not to harm CF. The contraction activity was then measured. A representative case of trypsin treatment was shown in Figure 1. All enzymes listed in the Table 1 displayed no reduction in CF activity. Thus, it was concluded that the protein component of CF is not responsible for induction of the muscle contraction.

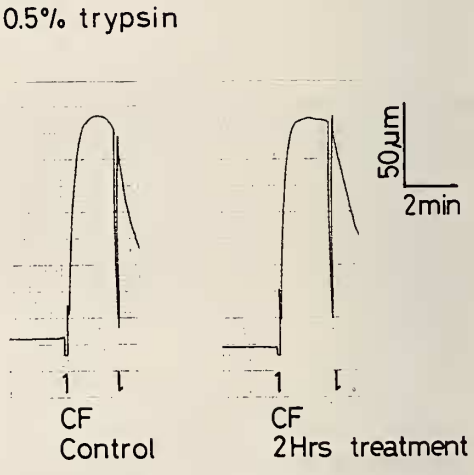


Fig. 1. No inhibitory effect of trypsin (5 mg/ml) on CF activity. Crude CF-I and trypsin were mixed for 2 hr at 37°C. The mixture was sometimes vigorously shaken. Trypsin was inactivated by boiling water, and CF activity was then measured.

The contraction activity in the carbohydrate component of the CF was examined. Cellulase and $\alpha$-glucosidase were used at the concentration of 1 mg/ml and were incubated with a crude extract of the aboral intestine (2.5 mg/ml) overnight at 37°C. As illustrated in Figure 2, both of these enzymes moderately reduced the contraction intensity. Moreover, the effect of neuraminidase (10 mU/ml) and RNase (1 mg/ml) on the contraction-inducing activity of the CF was investigated, but no inhibitory effect was observed. Thus, the possibil-

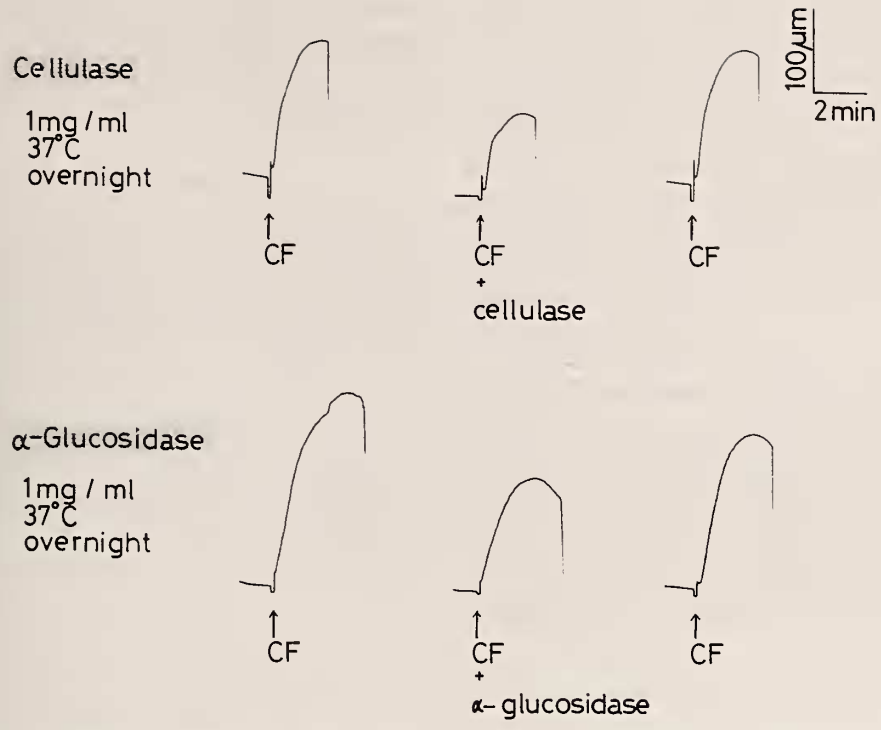


FIG. 2. Inhibitory effect of cellulase and α-glucosidase on CF activity. Incubation time between crude CF-I and glycolysis enzyme was performed overnight at 37°C. Glycolysis enzymes were inactivated by boiling water, and CF activity was then measured. Duration between the two contractions was 30 min.

TABLE 1. No inhibitory effect of proteolytic enzymes and others on CF activity

| Enzyme | Inhibition |
|---|---|
| trypsin | — |
| pronase P | — |
| papain | — |
| pepsin | — |
| carboxypeptidase A-PMSF | — |
| neuraminidase | — |
| RNase | — |

—, no inhibition.

ity that the carbohydrate component of CF might evoke the contraction of gonadal smooth muscles of the sea urchin was considered.

## DISCUSSION

In a preceeding paper [1], we described that CF is a heat stable glycoprotein. In the present paper, the carbohydrate component rather than the protein one in CF has been documented to possess the contraction activity. Accordingly, since polysaccharide usually is known to be resistant against heat treatment, a property of heat stability of CF appears to be responsible the contraction activity to its carbohydrate portion.

The present study using the sea urchin as invertebrates has dealt with the smooth muscle contraction subustance originating from digestive tract. Generally, in vertebrates many gut hormones are well known to indicate several biological activities including the contraction-inducing or-inhibiting activity of the smooth muscle. Those substances are grossly divided into three groups and those molecular weights are known to be as follows: group of gastrin such as gastrin (human: minigastrin, 1,647; big gastrin, 3,839), cholecystokinin (3,919) and caerulein (1,352), that of secretin

such as secretin (3,055), vasoactive intestinal polypeptide (3,381), gastric inhibitory polypeptide (5,105) and glucagon (3,485) and others such as motilin (2,700), substance P (1,348) and others such as motilin (2,700), substance P (1,348), somatostatin (1,638) and bombesin (1,619). All of these well-known and -investigated substances have the biological active property within their polypeptide. On the other hand, CF (about 3,800) is nearly similar in molecular weight to those of gut hormones. It is very interesting that the carbohydrate component (poly- or oligosaccharide) of the glycoprotein can induce smooth muscle contraction in the sea urchin. Thus, we have found a very rare phenomenon that poly- or oligosaccharide possesses the biological activity of contraction activity. At present, the study concerning contraction-inducing mechanism of the smooth muscle by CF is being carried out. In addition, the contraction induced by CF-I fraction were not completely inhibited by cellulase and $\alpha$-glucosidase. This might be the reason there exists another contraction factor within CF-I gel fraction. This point is also being examined at present.

## ACKNOWLEDGMENTS

The authors are grateful to Prof. Haruo Chino, Biological Laboratory, The Institute of Low Temperature Science, Hokkaido University, for his encouragement throughout this work. This work was partly supported by a Grant-in-Aid for a Special Research Project in the field of Biotechnology.

## REFERENCES

1 Takahashi, N., Sato, N., Ohtomo, N., Kondo, A., Takahashi, M., and Kikuchi, K. (1990) Zool. Sci. **7**: 861–869.

2 Palmer, L. (1937) Physiol. Zöol. **10**: 352–367.